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BULLETIN

OF THE

TORREY BOTANICAL CLUB

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Vol. 50 No. 10

BULLETIN

OF THE

TORREY BOTANICAL CLUB

OCTOBER, 1923

Resting spores of Empusa Muscae

BESSIE GOLDSTEIN

(WITH PLATE 19)

INTRODUCTION

The parasitic fungus, *Empusa Muscae*, like its host *Musca domestica*, has been found in most parts of the world—Europe, Africa, North and South America. Great numbers of flies are killed every fall in Europe and this country by this fungus. The possibility that such a natural parasite of the house fly as *Empusa Muscae* might be useful in combating its host has always made the study of this fungus attractive.

One of the problems which has attracted the most attention relates to the method of wintering of the parasite. Most of its congeners among the Entomophthoreae winter as some form of resting spore in the dead body of the host. So far the only report of such spores in *E. Muscae* was made by Winter in 1881. Brefeld, whose studies of the fungus and its relations to the fly are by far the most complete, concludes that *E. Muscae* does not form resting spores, disregarding Winter's observations as inconclusive.

Cohn, in 1885, was the first to study this disease of house flies in detail. He observed that flies dying of the disease attached themselves by their proboscides to window panes or other objects and here died of convulsions ("unter schweren Kämpfen"). The legs after death are spread out as in life and not curled under the body as is the case when death occurs naturally. The abdomen is swollen and often reflexed. He observed, too, that soon after death whitish rings of fungous

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material appeared between the dark segments of the abdomen and that this condition was soon followed by a discharge of conidia.

Cohn gave the name *Empusa* to the fungus and described it as a three-celled organism. On tearing the tissues apart, he found the swollen hyphal cell, which he called the root-cell, within the body; arising from this he observed the long tube- or stalk-cell—the conidiophore or second cell; the latter gave rise to a third cell or conidium. He was not able to find within the fly's body any mycelial growth like that characteristic of other fungi.

In 1870, Brefeld described a series of observations on the infection of the fly by the conidia of the fungus, on the germination of the conidia, and on the growth and development of the fungus within the host's body. Brefeld found that the conidia germinated upon the fly's body, and that the germ tubes were able to pierce the thin integument of the under side of the body directly. Here the germ tubes give rise to large cells. These large cells, feeding upon the fat-cells and body-juices of the fly, give rise by budding to other large cells. From these cells the conidiophores finally grow out, so that the insect's body appears to be completely filled with these hyphal tubes. In five or six days after infection the insect dies, after attaching itself by its proboscis to a surface. Each hyphal tube ends with the building of a conidium. The throwing off of spores lasts two days. The rest of the conidiophore, after the discharge of the conidium, disintegrates in the fly's body.

Thaxter's description of the vegetative development of the genus *Empusa* seems to accord with that given by Brefeld for *E. Muscae*. According to Thaxter, the germ tube of a conidium enters the host's body and there grows rapidly in the body-juices of the insect. It does not produce a mycelium, however. Short, thick cells are formed, which reproduce rapidly by budding and, finally, completely fill the host's body. Thaxter has given the name "hyphal bodies" to these short, thick cells.

The house fly disease, as has been commonly observed in this country and Europe, seems to become most evident and widespread in the fall of the year. The flies appear in early spring, become more and more plentiful during the summer and fall, and again disappear during the cold months. Statistical

data to confirm this view are so far lacking, but it seems to be a matter of common observation. No one records finding the fungus before July in this country.

Brefeld found that the conidia of *E. Muscae* were very short-lived, not living more than a few days. Not being able, during all his observations, to find any resting spores, sexual or asexual, he was puzzled as to how the fungus could live through the winter when there were no flies to pass on the infection. He came to the conclusion that the disease must be endemic in the South, where the flies are active throughout the winter, and that as summer comes on the disease travels northward. Brefeld concludes, on the ground of his own and Dr. Möller's failure to observe any form of resting spore either in Europe, Africa, or South America, that *Empusa Muscae* has lost the power to produce resting spores.

Olive succeeded in keeping the fungus, *E. Sciarae*, on a small fly, *Sciara* sp., under observation for a whole year, and in all that time did not observe the formation of resting spores at all. He notes that the spontaneous appearance of the fungus in the laboratory cultures of the fly as early as March renders Brefeld's hypothesis improbable for this case. Olive concludes that *E. Sciarae* probably lives upon the successive generations of the flies breeding in the dung of warm stables through the winter. The ability of *Empusa Sciarae* to grow and reproduce asexually, attacking one generation of flies after another without the intervention of a sexual stage or resting period, is evident from Olive's cultures maintained throughout the year.

Thaxter finds that Brefeld's idea of the fungus moving northward each year with flies from the South is refuted by his observations that the disease appears in the South in July and that it regularly makes its appearance in the North at the same date.

Lebert, in 1857, in his paper on the fungous disease of flies, shows several figures which Thaxter refers to as possible resting spores. These figures do not seem to me to be at all likely to be representations of the resting spores of *E. Muscae*.

Giard, in 1878, stated that the reason resting spores of *E. Muscae* had not been found was that they are not formed in flies that have died indoors and that they do not occur in the interior of the fly's body. He found irregularly rounded, thick-walled cells on the outside of the fly's body, which he believed

to be the resting spores of the fungus. Each spore contained a large globule of oil in its centre, and was surrounded by a layer of protoplasm. He added that these were seen by Brefeld, who considered them to be identical with conidia.

I have found such irregular cells on the exterior of the fly's body and believe them to be plasmolyzed or dried conidial cells or perhaps portions of the conidiophores which have become dried up or shrunken and appear thick-walled. I have found such cells, not only in the external mass of shrunken conidiophores of dried house flies which contain perfectly formed and rounded resting spores, but also in the external mass of conidiophores of very dry blue bottle flies attacked by *Entomophthora X* (Riddle), whose abdominal cavities contain fully developed, rounded resting spores with the characteristic bullate processes on their outer walls.

It is worth while to review Winter's observations more carefully. He reported that he found dead flies attached to a window, most of which showed only the conidial stage, and he identified these conidia as belonging to *E. Muscae*. Three of these flies contained a mycelium in their body cavities. From the hyphae rounded bodies arose either as lateral or terminal swellings. These rounded bodies upon maturing were colorless, rich in oil drops, and covered with a uniformly thick membrane. They measured 30–50 μ in diameter. Brefeld's only reference to Winter's observations is in a footnote; this states that Winter found resting spores in several flies and believed them to be the spores of *E. Muscae*.

It does not seem altogether improbable that Winter may have seen the resting spores of *E. Muscae*. However, since no cytological study of the fungus was made and since no drawings were given, his statements have remained under question.

Lakon, in his paper on the wintering of *E. Muscae*, concludes that, since Winter found the resting spores once, resting spores must exist in this fungus, although no one since Winter has been able to find them. He adds that it is well known that the disease can occur upon other related flies. He suggests the importance of searching for the resting spores on other species of flies which are supposed hosts of *E. Muscae*.

OBSERVATIONS

In September, 1921, I began a study of *Empusa Muscae* and had under observation great numbers of flies, many of which were attacked by the fungus and died of the disease. I fixed and embedded for cytological study both fresh material and some very old dry specimens of flies. These dried house flies were found sticking to walls and windows by means of their proboscides. The live flies, which I had under observation and which died of the disease, came from the same locality near Ridgewood, New Jersey.

The dried flies were put in Merkel's solution for two hours, followed by a diluted solution of Flemming's weak for twenty-four hours. The material was run up into paraffin in the usual way, and the sections were cut 5 μ thick and stained with Flemming's triple stain. The sections of two of these flies showed multinucleated, rounded, thick-walled spores in their abdomens.

Having found resting spores in these dried house flies, I immediately began to search about for other dried specimens. Several dried flies were found sticking by means of their proboscides to the white porcelain glass shades around the electric lights in the laboratory. Evidently in making transfers of the flies under observation from one glass chamber to another, diseased flies had escaped into the room, and had attached themselves to the glass shades. These dried flies showed further evidence of having died of the disease, since, between the segments of the abdomen, the thin membrane still projected as if it had been pushed out by the pressure of the conidiophores, and the segments of the abdomen were still somewhat apart. Upon sectioning and staining this material, the same form of resting spores was found again, although the material was not as good as the first.

In January, I secured a number of dried flies from house walls in the same locality, and resting spores were also found in this material. In dry specimens of flies secured about dwellings in New York City this year (1922) I have again found resting spores, some fully formed, and others in process of formation.

Frequently such old dry specimens show only traces of disintegrated, mycelial threads and are often filled with bacteria of

various sorts. Apparently resting spores are not always formed, but their occurrence in different lots of material, collected in the fall and winter of 1921 and 1922, shows that they are by no means rare.

The chief reason why investigators have commonly failed to find the resting spores is apparently their failure to examine old, dried up material, such as can be found attached to walls and windows throughout the winter. The resting spores are evidently most commonly, though not always, formed very late and as a final stage of the disease, after the body of the fly has begun to dry up.

The evidence is clear that the resting spores I find belong to *Empusa Muscae* and not to *Entomophthora americana* or any of the other entomophthorous forms that are said to occur in the house fly. All the flies studied, fixed, and sectioned are typical house flies. The flies sectioned show no traces of other fungi.

That the fungus is *Empusa Muscae* is definitely established by the fact that the conidia in all my sections which show them fully formed or in process of formation are the broad, multinucleated type characteristic of this species (FIG. 1). They do not resemble in the least the narrower and uninucleated conidia of *Entomophthora americana*. In none of my sections have I observed a single branching conidiophore. The conidiophores of *E. americana* are regularly branched. Many of the sections containing resting spores also show the multinucleated conidia and simple conidiophores of *E. Muscae* projecting between the abdominal segments.

Finally the insects were all found attached by means of their proboscides and not by means of rhizoids appearing at other regions of the body, as is the case with the other entomophthorous forms that are said to occur upon the house fly.

As described in the literature several forms of resting spores occur in the Entomophthoreae and they may be either sexual or asexual in origin. When they are of sexual origin, they are described as arising through the fusion of equal gametes or equal hyphal bodies. The terms encysted hyphal body, chlamydospore, and azygospore are used to designate resting spores of asexual origin.

The term chlamydospore is properly used to designate a resting spore formed by the rounding up of portions of the pro-

toplasm of a hyphal tube. This rounded mass of protoplasm becomes thick-walled and forms a resting spore.

The term chlamydospore might be applied in the case of the Entomophthoreae to resting spores originating in any one of at least four different ways:

1. The rounding up of the protoplasm in a hyphal body to form a rounded, thick-walled spore within the hyphal body.
2. The swelling up of the tip of a hyphal filament to form a terminal, thick-walled resting spore.
3. The formation of rounded, thick-walled spores at different points along the hyphal filaments.
4. The formation of rounded, thick-walled spores as buds arising either from a hyphal body or a hyphal filament.

A clearer and more definite use of terms is much needed for these various cases.

When the hyphal body itself becomes thick-walled forming an irregular thick-walled body the term "encysted hyphal body" alone should be employed.

The term azygospore has been used in the Entomophthoreae not alone to designate a resting spore arising without a preliminary fusion of cells. It has been used also for spores borne at the tips of hyphae, formed along the hyphal threads, or arising as buds from hyphal bodies or filaments. Resting spores arising in all these ways, it seems to me, are merely asexual spores of typical chlamydospore type.

In my opinion the term azygospore should be restricted to thick-walled resting spores arising from either of two sexual gametes which have failed to fuse. Such has been the use of the term in the mucors, where in many cases two gametes approach each other, but do not fuse. Each or only one of the gametes then forms a thick wall and becomes an azygospore.

The resting spores of *Empusa Muscae* are relatively large, thick-walled bodies. They are generally well rounded, and are multinucleated. They are approximately 30 μ in diameter. Oval spores generally measure 21 × 30 μ.

I have no evidence to show that a pairing or fusion of nuclei takes place within these resting spores, although in many spores the nuclei do appear to be arranged in pairs. There is no evidence of either a reduction or an increase in the number of nuclei contained within a young spore as compared with a more mature

spore, such as Vuillemin describes for *Entomophthora gleospora*. The younger, thin-walled spores contain about the same number of nuclei as the very thick-walled spores.

Oil globules are present in the older spores. These usually occur as a few large drops. There may be a single large oil drop present, so that the protoplasm appears to be pushed to one side of the spore, and the nuclei are then grouped closely together in the denser mass of protoplasm. Generally the nuclei are scattered rather irregularly through the protoplasm. In the young spores, the protoplasm is more or less homogeneous and fine granular threads connect the nuclei (FIGS. 6 and 7).

The resting spores show an outer thin wall or membrane around them, which is probably the old hyphal wall. Inside this is the thickened wall. This wall is of an even thickness around the entire spore and is quite smooth even in mature spores. The wall appears to be composed of a single layer, and probably grows in thickness by deposition of material from the outer region of the protoplasm. Within the thick wall is the rounded protoplasmic mass, which may be only very slightly withdrawn from the thick wall, or very much so, the latter condition being due probably to poor fixation (FIG. 5).

The resting spores are obviously asexual in origin. They are chlamydospores in the strict sense. They arise most commonly at the tips of short hyphae or hyphal fragments (FIGS. 3 and 4) or by the division and rounding up of the protoplasm in the hyphal filaments themselves (FIG. 7). In both fresh and stained material, I have recently found some evidence that they may arise as buds on hyphal fragments or hyphal bodies (FIGS. 2 and 9).

The hyphal cells, at the end of the nutritive period of the fungus, give rise to long hyphal tubes which grow out toward the body walls of the host. These hyphal tubes are non-septate and contain many nuclei. They are always unbranched. Many reach the body wall of the insect, and travel along parallel to it, until the thin membrane between two segments of the abdomen is reached. Here, with other tubes that have reached this region directly, they push out the membrane and finally grow out to the exterior. At the tip of each conidiophore, a conidium arises as a bud and, when mature, is finally cut off by an ingrowing wall. The conidium is forcibly abjected by the bursting of the upper swollen region of the conidiophore.

The remainder of the conidiophore tube, as Brefeld also observed, disintegrates. The collapsed, shrunken, and disintegrating hyphal tubes can be seen filling the openings between the segments of the abdomen and projecting a little from the body in sections of flies in which the conidiophores have already discharged their conidia. Great numbers of bacteria are always present feeding upon the contents of the disrupted hyphae.

After the discharge of conidia, there still remain within the abdominal cavity numerous hyphal filaments and other hyphal fragments which have not been able to reach the exterior. As long as suitable moist conditions are present, these hyphal tubes remain alive and unaltered and are later able to force their way between the collapsed conidiophores to the exterior to form and discharge their conidia. If, however, the weather is dry, the development of conidia may be checked, and it is probably under these conditions that the resting spores are formed.

The terminally formed resting spores arise as swellings in the terminal regions of hyphal tubes and are finally cut off by cross walls (FIG. 4). Within the hyphal membrane the heavy wall is formed and a somewhat pyriform or more or less oval resting spore results. Those resting spores which are formed along the hyphal tubes are generally more rounded in form. I have found two, three or four round, thick-walled spores occurring in a chain, connected by the collapsed and emptied hyphal tube membrane in which they arose (FIGS. 5 and 7). Not all the resting spores are round or oval. Some are simply portions of the hyphal tube which have become only slightly more swollen than the original width of the hyphal tube.

Resting spores are found only in the abdominal cavity, although traces of vegetative mycelial threads can be seen in the thorax and head of the insect. The germ tube first makes its entrance into the abdominal cavity as Brefeld found, and the fungus passes most of its vegetative state feeding upon the body juices and soft tissues which are found in this region.

SUMMARY

Resting spores are formed in the life cycle of *Empusa Muscae*. Failure to find them on the part of Brefeld and other students is apparently because they are formed very late in the development of the fungus.

These resting spores are in my opinion to be regarded as chlamydospores, formed under dry conditions after conidial discharge is no longer possible. They are formed most frequently as terminal swellings on short hyphal fragments or tubes, or intercalary in the hyphal filaments, or by apparent budding from hyphal bodies. They are generally rounded cells, with smooth, evenly-thickened walls, and contain many nuclei.

I wish to gratefully acknowledge my indebtedness to Professor R. A. Harper for his assistance and advice in this study and in the preparation of this paper.

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Explanation of plate 19

The drawings were made with the aid of the Abbé camera lucida, from sections 5 μ thick and stained with Flemming's triple stain, except figure 8 which is a drawing from fresh material mounted in lacto-phenol.

A Zeiss microscope was used with a 1/12 inch oil immersion objective and a number 3 ocular. The magnification is about 1200 diameters. FIG. 8 was drawn with the Zeiss objective D and number 3 ocular.

FIG. 1. A broad multinucleated conidium characteristic of the species.

FIG. 2. A young resting spore which probably arose by budding from the tip of a hyphal fragment.

FIG. 3. A young resting spore formed at the tip of a hyphal tube.

FIG. 4. A young chlamydospore cut off from the tip of a conidiophore.

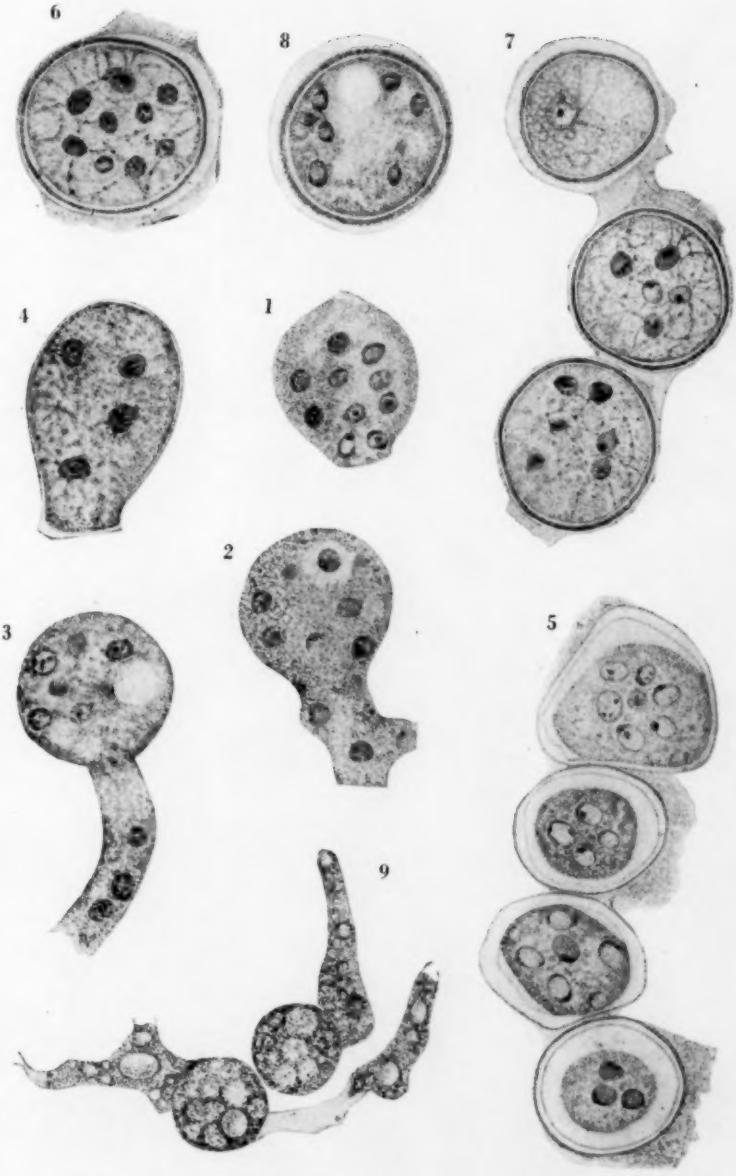
FIG. 5. A chain of four young spores. This material was not fixed well and shows considerable shrinkage. The protoplast in each spore is drawn away from the inner membrane, which is itself shrunken away from the spore wall. The outer thin membrane does not show and is probably closely applied to the slightly thickened spore wall. I am not certain that the uppermost and irregularly shaped spore belonged to the same hypha. This spore may have arisen from another hypha which lay across the first.

FIG. 6. A chlamydospore showing numerous nuclei and granular threads connecting them.

FIG. 7. A chain of three chlamydospores connected by the old conidiophore tube wall.

FIG. 8. A more mature resting spore.

FIG. 9. Two young rounded spores at the tips of hyphal filaments which, as in FIG. 2, probably arose by budding. The protoplasm contains many oil drops.



GOLDSTEIN: RESTING SPORES OF EMPUSA

An ecological study of Cheilanthes gracillima

F. L. PICKETT

(WITH THIRTY-THREE TEXT FIGURES)

Cheilanthes gracillima D. C. Eaton is found in the mountains of the Pacific Coast region from British Columbia to Mexico. It is usually found at altitudes of 5000–7000 feet, and more rarely as low as 1500 feet. J. H. Sandberg* reported finding this fern in Idaho on "mountains near Viola, Latah County, June 26 (1892)." Later surveys have located it over most of the range of Thatuna Hills, extending from Latah County in Idaho westward and northward into Whitman County, Washington. These "Hills" are old mountain peaks composed of granite and quartzite rocks with an altitude of 3000 to nearly 5500 feet. They stand out in the landscape as more or less isolated peaks or buttes, rising 1000–2500 feet above the great lava plateau of the Palouse Country, as the plains of southeastern Washington are called.

Throughout its range *C. gracillima* is an inhabitant of rocky, exposed ledges, and consequently has but a limited water supply. In the southern part of its range climatic conditions combine with habitat to reduce the amount of available water, so that this *Cheilanthes* has come to be known as a typical xerophytic species. The isolated buttes in Whitman County, Washington, are subject to a combination of severe conditions. The total rainfall of about twenty-four inches is limited almost wholly to the period between the first of October and the last of May. Throughout the summer almost constant winds with a very low humidity prevail. The temperature range through the year is from $-20°$ to $+110°$ F. Add to these conditions the fact that *C. gracillima* grows only in the crevices of otherwise bare rocks, with southern or southeastern exposure, with no other protection than an occasional dwarf specimen of *Pinus ponderosa*, and it is at once evident that some striking characteristics of structure and development must be present to make possible the vigorous growth of this fern in this locality. The fern is fairly common, however, and does show normal, vigorous plants.

* See Holzinger, Contr. U. S. Nat. Herb. 3: 267. 1895.

FIELD AND LABORATORY INVESTIGATION

Examination of *C. gracillima* at different seasons of the year shows the following items aside from the developmental history, which is given later. The most vigorous growth is in the spring, March to May. Fronds are persistent through the summer and to some extent into the second summer. With the advent of summer and the reduction of both atmospheric and soil moisture the fronds and individual pinnae are closely curled together. They take on a blue-gray appearance, and are harsh to the touch, like herbarium specimens. The rootstocks are buried under a few inches of light soil in the rock crevices but are quite dry in midsummer. The roots are small, fibrous, covered with a definite corky layer, and extend sometimes as far as four or five feet through the crevices in the rocks. An abundant growth of new roots is produced by the rootstocks each spring.

On August 10, 1921, mature, fertile fronds were collected for cultural work. Although mature, these fronds had lost but few, if any, of their spores when collected. They were placed in paper folders and stored in the laboratory. When the folders were opened on November 27, but very few loose spores were found, and examination showed the sporangia unopened, almost without exception. After being in water for an hour some fronds were placed on filter paper in a desiccator over night. When examined the next morning the paper was well covered with spores. By repeating this process three or four times it was possible to empty nearly all the sporangia.

On November 28, 1921, several cultures were started by sprinkling spores over dishes of Knop's solution, one-half usual strength, over dishes of agar agar made up with one-half strength Knop's solution, and over sterilized soil in perforated clay saucers. The spores germinated freely after ten to twelve days, and by December 17 germination had proceeded far enough for the cultures to show a distinct green surface. Differences of temperature through a fairly wide range, 45°–70° F., seemed to make no appreciable change in the rate or percentage of germination.

The spores of this plant are nearly spherical with their clearly marked sutures between pairs of thickened, rounded ridges FIG. 1). They show marked variability as to size, measuring

425–600 μ in greatest diameter. The exine is very hard and brittle. Slight crushing under a coverglass regularly results in such fractures as shown in FIGS. 2, 3 and 4, and the germination of well-matured spores results in the same type of irregular fracture. Spores which are immature, as shown by their light color and thinner exine, may produce normal plants, but the germination of such spores usually results in the separation of exine-segments along the sutures. The spores are uninjured by

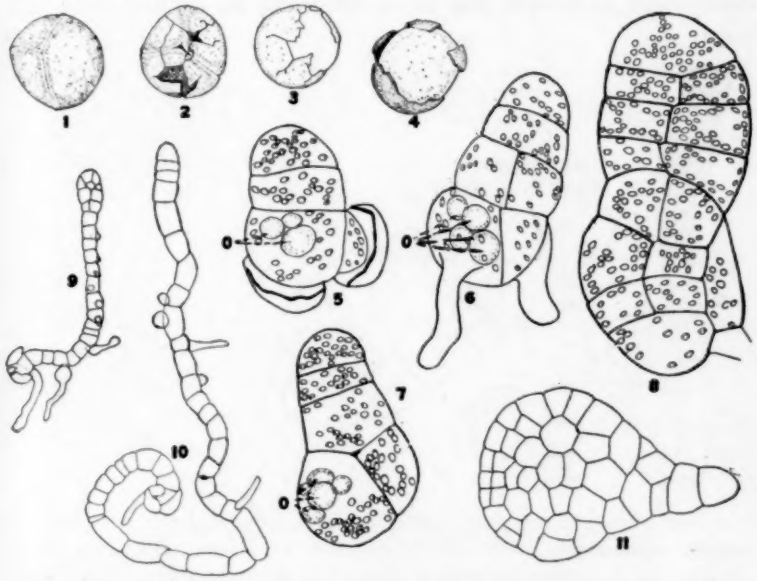
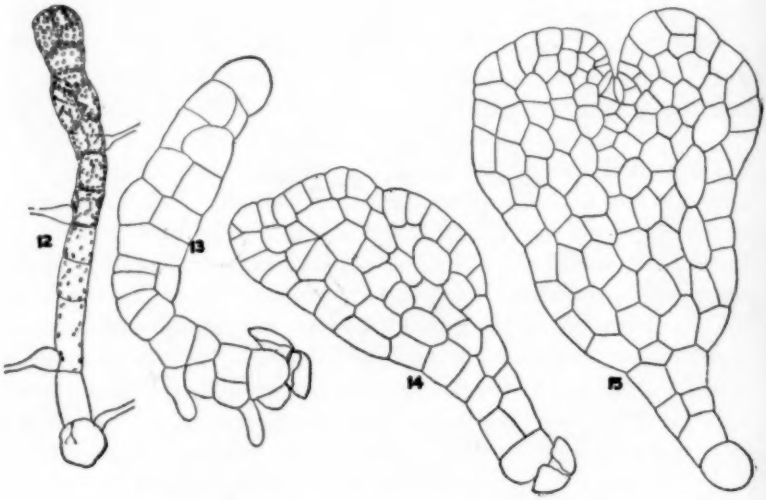


FIG. 1. A normal mature spore. FIGS. 2–4. Spores showing typical fracture of the exine under pressure. FIGS. 5–8. Young sporelings showing distribution of oil-globules, O, and chloroplasts. FIGS. 9, 10. Unusually filamentous forms of antheridial plants, the second having developed under water. FIG. 11. A normally developing archegonial plant showing unusual symmetry.

desiccating influences. Fronds placed in desiccators over anhydrous calcium chloride in November, 1921, remained there until March, 1923, and then furnished spores nearly all of which were capable of germination. A number of interesting questions enter at this point. It is not desirable to take them up in detail at this time; but for other investiagtors who may wish to know more of details it should be said that the desiccators used were

of standard, Scheibler type, non-tubulated, with well-ground joints sealed with a film of vaseline, having a total capacity of about 2 liters and containing 300 grams of freshly fused $CaCl_2$.

Microscopic examination of spores in sections made of pinnules taken from the desiccator, plunged directly into chloroform or benzole and then imbedded in paraffine, showed the cytoplasm dry and shrunken to the sides of the spore. In general external appearance the spores were the same after desiccation as before, the exine retaining its normal spherical form.



FIGS. 12, 13. Elongated forms produced in reduced light. FIGS. 14, 15. Normally developing archegonial plants.

The germination of the spores and the development of the prothallia show some unusual features. A protonemal chain of from two to ten cells is regularly formed before the oblique wall cutting off the first apical cell appears, as shown in many of the figures. Especially abundant in cultures exposed to bright light were plants almost or entirely lacking this protonemal development (FIGS. 5–8). The spores when first germinated show several drops of oil, and this is evident after several cells have been formed (FIGS. 5–7). Very quickly after germination the cells of the spore and succeeding cells show abundant chlorophyll (FIGS. 5–8). The prothallia are strictly dioecious and show

marked differences in development. Archegonial plants are nearly symmetrical (FIGS. 11, 15), with a well-developed median thickening, and may reach an extreme length of 2 mm. Antheridial plants are much smaller, very irregular in form, without median thickening, usually less than 0.5 mm. in length and sometimes filamentous (FIGS. 9, 10), and may have less than twenty cells when the first antheridium appears. So small are the antheridial plants and so irregular in shape that they may be overlooked in a casual examination of a soil culture.

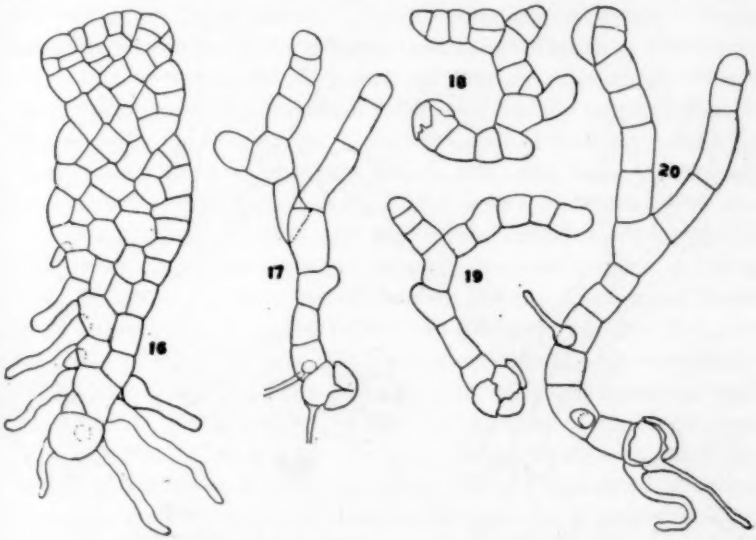


FIG. 16. Normal prothallium showing resumption of growth over wide apical area after period of drought. FIGS. 17–20. Sporelings showing tendency for proliferation through multiple growing points.

The author has elsewhere discussed at length the extreme sensitiveness to changing light intensity shown by other fern prothallia.* The young prothallia or sporelings of *C. gracillima* show much of the same sensitiveness. This is well shown by FIGS. 11, 12, 13, 14 and 16, in which FIGS. 11, 14 and 16 are of sporelings grown on Knop's solution in Petri dishes near the laboratory window, and FIGS. 12 and 13 those of plants from

* Some ecological adaptations of certain fern prothallia—*Camptosorus rhizophyllus* Link., *Asplenium platyneuron* Oakes. Am. Jour. Bot. 1: 477–498. *pl. 49, 50 + f. 1–19.* 1914.

similar cultures shaded by the first, which were immediately above them. Exchanging the positions of these dishes made sufficient change in light intensity to produce marked changes in the growth of the sporelings. Very nearly the same peculiarities of form appeared when plants were allowed to develop under water. FIGS. 10 and 25 represent extreme cases of irregular development under water. These, being antheridial

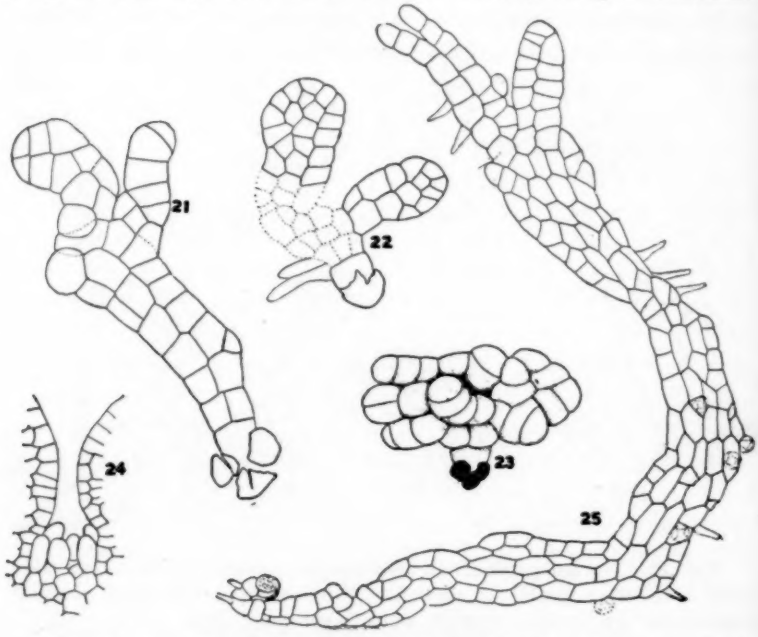


FIG. 21. Sporeling showing tendency for proliferation through multiple growing points. FIG. 22. Marginal proliferation following injury to young plant; dotted lines show injured cells. FIG. 23. Unusual production of growing tips in three or more planes. FIG. 24. Unusual growth of marginal cells in sinus. FIG. 25. Greatly elongated antheridial plant developed under water.

plants, would be expected to show irregular growth, but they are much more attenuated than plants grown on the surface of culture fluid or on soil. FIG. 25 is that of a plant developed wholly under water in normal light.

The soil cultures grew nicely up to April, 1922. At that time some cultures were flooded and within a month showed abundant sporophyte growth. Other cultures were allowed to

become entirely dry after April 1, 1922. This condition continued up to July 31, 1922, when one of the cultures was moistened from below. The plants in this culture showed complete recovery the following day. This culture was not moistened again until November 6, when it again showed complete recovery. On September 21, a second culture which had received no water since April 1 was moistened from below and on the following day this culture showed complete recovery. From these experiments it was evident that the prothallia of this plant were able to withstand very unusual drought conditions. Steps were taken to determine more fully their ability to survive such treatment. On November 7, portions of soil bearing prothallia were taken from a culture, which had not been moistened since April 1, and placed in two desiccators, one containing anhydrous calcium chloride and the other anhydrous phosphoric acid (sticks). From time to time shall portions of these cultures were removed and were moistened by placing them upon moist filter paper. For a considerable time this was repeated in forty-eight hour periods until it became evident that the extreme desiccation was doing but little damage, after which the periods were lengthened. On January 29 the last portion of these cultures was removed. Its plants showed almost complete recovery within a few hours after being moistened. These prothallia had thus survived exposure to absolute drought for a period of thirteen weeks. We have no reason to believe any moisture could have reached them in any way during that time. The soil on which they were growing was a small cake, perhaps 10 sq. cm. in total extent in the beginning and at no place exceeding 5 mm. in thickness; moreover, this soil was taken from a culture which had been exposed to normal dry atmosphere through a period of seven months before being placed in the desiccator. The same type of apparatus was used as described above, and the same precautions observed to prevent entrance of invalidating factors.

Final proof of the survival of plants was made by placing them under normal conditions of growth for a considerable period. In every case where they have been reported as surviving the desiccation, they continued to grow and even produced sporophytes. In the case of plants which had already produced sporophytes before being placed in extreme conditions it was found that prothallia and sporophytes survived equally well.

As in the case of spores, so prothallia and young sporophytes show no evidence of ability to *resist* desiccation. Vigorous plants on moist soil when exposed to the warm, dry atmosphere of a class room were completely wilted in thirty to forty minutes. The peculiar characteristic of this, as of other ferns studied by the writer, seems to be the ability to survive the actual loss of water far below the percentage usually considered essential to the preservation of life.

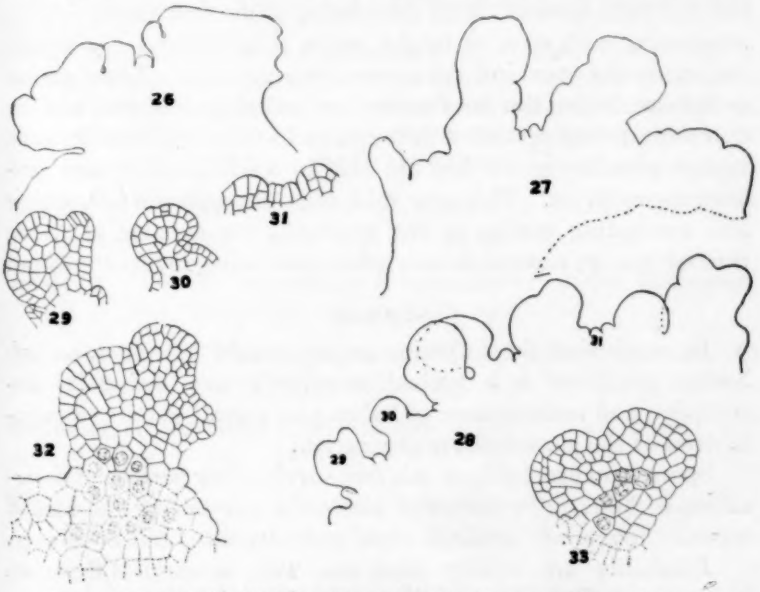
As far as the writer has been able to find there is no previous record of the survival of such extreme desiccation by fern prothallia or sporophytes. Attention has already been called to the importance of such ability to survive difficult conditions in the preservation of two other species of ferns, *Camptosorus rhizophyllus*, and *Asplenium platyneuron*. No doubt, in the case of *C. gracillima*, this peculiarity is an important factor, if not indeed the most important, in making possible the growth of this fern on the buttes of southeastern Washington, and in the even more arid regions in the southern portion of its range.

In December, 1922, experiments were carried on to determine the ability of these prothallia to withstand low temperatures. Dry prothallia were uninjured by an exposure of two weeks to temperatures ranging from 35° F. to — 10° F. Cultures of vigorous growing plants on moist soil showed considerable injury through exposure to the same conditions, although a few plants survived. The finding of a small number of prothallia on Kamiak Butte in April, 1923, prothallia too large to have developed this year, is evidence that they do survive the winter-conditions in this locality.

Seemingly closely connected with the hardy qualities of other ferns studied, is the tendency to develop outgrowths on old prothallia. This form of vegetative propagation is commonly found in old cultures of *C. gracillima*. As already mentioned various marginal or submarginal groups of cells, in no way related to the apical sinus or group, renew their meristematic characteristics and produce various forms of outgrowths. This tendency is more strongly developed in *C. gracillima* than in other forms studied. FIGS. 26, 27 and 28 show margins almost wholly given over to this peculiar growth. In old cultures prothallia sometimes show a marked crenulate and ruffled appearance produced by the abundance of these outgrowths.

Even young plants show much more ready branching, leading to the formation of plural growing areas, than is usual, as shown by FIGS. 17, 18, 19, 20, 21, 22 and 23. The plants in FIG. 22 had been injured before the two outgrowths were formed, that in FIG. 23 was grown under normal conditions on the surface of a culture solution.

Another peculiar type of proliferation was observed. In old archegonial prothallia grown without the possibility of fertilization, marginal cells in the sinus showed the unusual size and arrangement illustrated in FIG. 24. This, as well as some other



FIGS. 26–28. Marginal proliferation of old prothallia. FIGS. 29–33. Cellular details of marginal growths; the small numbers on FIG. 28 show the position of FIGS. 29–31.

peculiar structures on old archegonial prothallia, suggests a type of apogamous embryo development; but no embryo that could be definitely assigned to apogamous origin has as yet been observed in this species.

These outgrowths in general behave as normal prothallia, producing rhizoids, antheridia and archegonia. As might be expected, the monosexual character of the old prothallia is carried into these outgrowths. The formation of marginal

structures is so like the development of new tissue by injured liverworts as to be quite striking. FIGS. 29–33 show various types.

From work so far done the seasonal history of *C. gracillima* is as follows:—In the early spring sterile fronds are produced. These are followed in June by fertile fronds. The spores are matured before the winter's moisture has entirely left the scant soil in the rock crevices where the fronds grow. Aside from periods of very slight showers the fern remains inactive until late summer or early autumn. The fronds become dry and curl up closely, but, contrary to the usual habit of ferns, they do not lose their spores. With the coming of the first autumn rains, alternating with days of bright, warm sunshine and dry winds, the sporangia open and the spores are scattered. These spores germinate during the brief periods of sufficient moisture and lie quiescent during periods of drought, and so through considerable, broken growth-periods they are able to reach maturity and produce sporophytes. This may take place through the fall, winter and succeeding spring, or the prothallia are capable of living through one dry summer season before producing any sporophytes.

SUMMARY

In conclusion the following points should be noted. *Cheilanthes gracillima* is a typical xerophytic fern, owing its distribution and maintenance of habitat to a great extent to peculiarities of the gametophyte generation.

Spores and prothallia of this fern survive long periods of desiccation without any evidence of means for preventing the loss of water. Spores are retained after maturity until fall or winter.

Prothallia are strictly dioecious with marked differences in form and size of antheridial and archegonial plants.

Prothallia survive the usual winter conditions of eastern Washington. Marked development of vegetative propagation of prothallia is shown.

All figures used are from camera lucida drawings. FIGS. 26–33 are from drawings by Miss Lotta Dueber, a graduate fellow in the Botany Department for the year 1922–23. Miss Dueber should also be given credit for her care of cultures during that year. The other figures used are from the author's drawings.

THE STATE COLLEGE OF WASHINGTON,
PULLMAN, WASHINGTON

INDEX TO AMERICAN BOTANICAL LITERATURE

1923

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Reviews, and papers that relate exclusively to forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included, and no attempt is made to index the literature of bacteriology. An occasional exception is made in favor of some paper appearing in an American periodical which is devoted wholly to botany. Reprints are not mentioned unless they differ from the original in some important particular. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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